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Assessment of sex differences and amphetamine on schedule-induced polydipsia

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Assessment of sex differences and amphetamine on schedule-induced polydipsia

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ABSTRACT

ASSESSMENT OF SEX DIFFERENCES AND AMPHETAMINE ON SCHEDULE-INDUCED POLYDIPSIA

By

Min-Kyung Park

Amphetamine (AMPH) is one of the most common psychotropic drugs abused in the United States. Its major pharmacological effect is to increase synaptic dopamine levels in the mesolimbic reward pathway, which in turn causes behavioral effects in animals, and subjective effects in humans. These reinforcing properties of AMPH trigger very strong levels of craving the drug, and eventually result in patterns of compulsive use of AMPH. Regarding psychostimulant action, female rats have been reported to be more vulnerable to the reinforcing effects of psychostimulants. In the current study, schedule-induced polydipsia (SIP), an animal model of compulsive behavior, was applied for the further study of sex differences in the behavioral effects of AMPH. SIP is a phenomenon whereby food-restricted rats exhibit exaggerated polydipsic behavior when presented with food pellets under an intermittent schedule of reinforcement. This behavior appears to be mediated by the neurotransmitter dopamine in the brain's limbic system, and this neurotransmitter and system is also affected by psychostimulant drugs. During the SIP training sessions, it was found that female rats needed more sessions to develop stable schedule-induced polydipsic behavior. In line with previous studies, AMPH dose-dependently decreased total water intake and licks during a SIP task. Significant differences were found on their total number of lever presses and reinforcers earned.

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LIST OF ABBREVIATIONS

SIP = Schedule-induced polydipsia

AMPH = Amphetamine

OCD = Obsessive-Compulsive Disorder

INTRODUCTION

Schedule-induced polydipsia (SIP) refers to an excessive drinking behavior induced by a periodic delivery of reinforcers, but not is directly reinforced by them. There is a considerable amount of literature with respect to the neurobehavioral and neurochemical mechanism of schedule-induced polydipsia, and it appears that dopamine activity is responsible for creating schedule-induced drinking. Many different categories of drugs have been widely studied to examine their effects on schedule-controlled behavior and schedule-induced behavior under the adjunctive polydipsia model; however, the knowledge of AMPH-induced behavioral outputs in preclinical studies is restricted to male rats. That said, the sex of rodent models in schedule-induced polydipsia research has been biased to male dominant models. Therefore, this paper included both male and female rats as to examine sex differences while the animals developed polydipsic behavior during the experimental procedure. Behavioral responses to AMPH in female rats compared to male rats were also investigated in light of previous work established on the topic of sex differences in addiction. This paper was formatted using the APA guidelines.

LITERATURE REVIEW

Behavior is typically classified into two categories: respondent and operant conditioning (Wetherington, 1982). However, Falk (1961) introduced a new behavioral phenomenon which does not belong to either respondent or operant conditioning. Falk (1961) reported that food-deprived rats, which were trained to press a bar on a 1-minute variable interval schedule, exhibited a very large magnitude of water consumption behavior (amounting to half of their body weight during a 3.5-hr session) immediately after the food was presented as a reinforcer. This phenomenon drew great attention from physiologists and psychologists of the day, because it cannot be explained by a regulatory account of drinking (Kissileff, 1973; Toates, 1979). This psychogenic polydipsia eventually led to the establishment of a third class of behavior - adjunctive behavior (Wetherington, 1982).

Adjunctive behavior is thought to be induced by long interval parameters, where these schedules of reinforcement are originally expected to serve as reinforcing stimuli for increasing the likelihood of another class of behavior (Falk, 1977). Specifically, 'induced' means that these adjunctive behaviors emerge incidentally as a "side effect" of the predominant controlling variables of such familiar targeted behaviors as bar-pressing or key-pressing behavior (Foster, 1978, p. 45). However, excessive behaviors with reliable form, but adjunctive, persistently occur by exposure to certain schedule parameters. That is, adjunctive behavior can be defined as an unreinforced behavior which is contrarily maintained at a high probability after the presentations of reinforcing conditions on an intermittent schedule of reinforcement.

Since the discovery of adjunctive behavior by Falk (1961), a wide variety of responses in terms of adjunctive behavior were observed with various species, schedules, and reinforcers (Falk, 1977; Porter, Brown, & Goldsmith, 1982; Reid & Dale, 1983). As reported by Haight (1989), these response of patterns vary among different animals, yet are consistent within individuals. According to Foster (1978), prototype studies of animals in the context of adjunctive behavior have reported the demonstrations of abnormal, exaggerated adjunctive behavior including eating (Bellingham, Wayner, & Barone, 1979), drinking (Falk, 1961), wheel-running (Riley, Wetherington, Delamater, Peele, & Dacanay, 1985), air-licking (Mendelson & Chillag, 1970), aggression (Looney & Cohen, 1982), and escape (Azrin, 1961; Brown & Flory, 1972). Schedule-induced polydipsia (SIP) aforementioned is also categorized as an adjunctive behavior.

There are some experimental requirements for the development of a typical SIP. Rats should be food-restricted to 80-90% of their normal free-feeding body weight, so that the organisms could be in a "deprivation state" of food (i.e., reinforcer) (Ford, 2014; King, 1974, p. 325). It is also imperative that rats' bar-pressing performance should be conditioned on the schedule of intermittent food delivery (Ford, 2014). Another necessary condition for the generation of SIP is that rats should be given small quantities of food (Falk, 1969). This condition might provide valuable insight into the underlying mechanism of SIP.

The fundamental mechanism of SIP is still unknown; however, the prevailing hypothesis suggests that SIP occurs as a coping mechanism for frustration or arousal generated by a stressful situation where rats' hunger drive is thwarted by the presentation of substantially lower amount of food pellets than normal (Brett & Levine, 1979, 1981; Lotter, Woods, & Vaselli, 1973; Thomka & Rosellini, 1975). This elaboration of the SIP mechanism is supported as rats

decreased the level of SIP with increasing meal size (Falk, 1967). This hypothesis has been further supported by the findings that polydipsic rats also exhibited reduction of blood corticosterone levels during the course of a SIP regime (Dantzer, Terlouw, Mormede, & Le Moal, 1988). Thus, SIP development may reflect on clinically maladaptive coping mechanisms in response to the intrinsic levels of anxiety represented as the development of obsessive-compulsive disorder and, in a broad sense, drug addiction (Flores et al., 2014; Gilpin, Badia, Elder, & Stewart, 2008; Levine & Levine, 1989; et al., 2011; Piazza, Mittleman, Deminière, Le Moal, & Simon, 1993). Hence, SIP has been proposed as a behavioral model of drug abuse wherein responsiveness to stress is a common indicator to vulnerability to drug dependence (Ford, 2014; Khantzian, Mack, & Schatzberg, 1974; Liu et al., 2005; Shen, Wang, Wan, & Tung, 2001; Tarter, 1988; Weiss, Imperato, Casu, Mascia, & Gessa; 1977).

In fact, stress and drugs abuse share the property of increasing extracellular dopamine levels in limbic brain regions (Sinha, 2008). As discussed above, the reinforcing properties of drugs abuse involve their activation of the mesolimbic dopaminergic pathways, which includes dopamine neurons originating in the ventral tegmental area and extending to the ventral striatum and the prefrontal cortex (Chiara & Imperato, 1988; Spanagel & Weiss, 1999; Pierce & Kumaresan, 2006). However, stress exposure and increased levels of glucocorticoids also enhance dopamine release in the nucleus accumbens (Dunn, 1988; Piazza & Le Moal, 1996; Thierry, Tassin, Blanc, & Glowinski., 1976). Accordingly, synaptic adaptation can be triggered as a consequence of the activation of the mesolimbic pathways by stress and drugs abuse (Cleck & Blendy, 2008; Liston et al., 2006; Saal, Yan, Bonci, & Malenka, 2003).

In a similar vein, a considerable body of evidence have implicated dopaminergic pathways as the neurochemical basis of the occurrence of SIP (Mittleman, Rosner, & Schaub,

1994; Wallace, Singer, Finlay, & Gibson, 1983; Weissenborn, Blah, Winn, & Phillps; 1996). For instance, Weissenborn et al. (1996) measured the release of dopamine in the nucleus accumbens (NAC) throughout the SIP sessions, and discovered that dopaminergic activity within the NAC mediates the acquisition and emission of schedule-induced drinking. Secondly, a substantially decreased amount of water in an SIP procedure was reported with bilateral 6-OHDA lesions of the NAC (Wallace et al., 1983). The 6-OHDA lesions of the lateral septum, which is the main dopaminergic brain structure of the NAC, caused the destruction of dopamine neurons. As a result of inhibited dopamine signaling in the NAC, dopamine depletion of the nucleus accumbens septum occurs. Therefore, decreases in SIP were observed. Moreover, water consumption was significantly reduced in polydipsic rats following the manipulation of dopamine D1 and D2 receptor agonists and antagonists (Mittleman, Rosner, & Schaub, 1994). This findings supported the notion that dopaminergic neural transmission plays a pivotal role in causing behavioral change in the established SIP paradigm.

There is now a wealth of evidence suggesting that some basic biological differences exist between males and females in every phase of the addiction cycle (Lynch, Roth, & Carroll, 2002; Carroll, Lynch, Roth, Morgan, & Cosgrove, 2004). This is true not only for most illicit drugs, but also for alcohol or even for gambling (Bobzean, DeNobrega, & Perrotti, 2014; Tavares et al., 2003). For example, women exhibit accelerated escalation patterns from initial casual drug taking to addiction (Becker, 2016; Becker, McClellan, & Reed, 2017). Furthermore, women experience more intense withdrawal symptoms than men after a certain period of time of abstinence (Hogle & Curtin, 2006; Hudson & Stamp, 2011; Becker & Koob, 2016).

These findings have been corroborated by preclinical studies in rodents. In the test for self-administration of psychostimulants, female rats exhibited a greater initial response to the same dose of drugs than males by working harder to get a single injection (Cummings et al., 2011; Lynch & Taylor, 2004; Reichel, Chan, Ghee, & See, 2012). Female rats also tend to show greater relapse susceptibility when exposed to stressful conditions or drug-associated cues (Anker & Carroll, 2010; Becker, 2016; Becker et al., 2007). Thus, in the laboratory, female rats acquired their drug dependence more rapidly than males. So too, female rats presented greater motivational behaviors, which, in turn, led to further behavioral problems with craving and relapse.

These sex differences in addiction have been suggested to be due to circulating ovarian hormones in animals and humans (Jackson, Robinson, & Becker, 2006; Lynch, 2008). In particular, higher levels of estradiol (estrogen) seem to be associated with drug taking and quitting behavior (Becker, 2016; Becker et al., 2017). For example, women tend to experience more intense subjective effects of the drug during the follicular phase when estradiol is elevated relative to the luteal phase of the menstrual cycle when both estradiol and progesterone increase (Evans, Haney, & Foltin, 2002; Justice & de Wit, 1999, 2000; Li & Graham, 2017). In rodents, ovariectomized rats who were given estradiol exhibited enhanced motivation for drug taking, and took more psychostimulant(s) (Becker & Hu, 2008).

Although substantial progress has been made with regard to neural and psychological mechanisms underlying sex differences in addiction, more extensive studies are needed at the behavioral level in the female, where historically sex- or gender-related differences in addictions have been understudied. In this study, research interests centered on providing further

information regarding sex differences on AMPH manipulations, particularly in the interaction between dosage and environmental context alongside schedule-induced polydipsia (SIP).

In the experimental paradigm of SIP, three behavioral patterns are shaped into a sequence of responses within the interreinforcement intervals: terminal (*i.e*., schedule-dependent), interim (*i.e*., schedule-induced), and facultative behaviors. These three different types of behavior are identified based on their contingencies relating to food reinforcement and by their temporal distributions in interreinforcement intervals (Pellón & Blackman, 1992; Staddon, 1977). In rats, these behaviors correspond to bar pressing, licking, and locomotion, respectively (Reid & Staddon, 1990).

Based on the model of SIP, AMPH appears to reduce the reactivity of response to the stress induced by SIP by suppressing water intake in rats (Brett & Levine, 1979; Didriksen & Christensen, 1994; Liu et al., 2005; Segal, Oden, & Deadwyler, 1965; Robbins, Roberts, & Koob, 1983). Some authors suggested that this behavioral effect of AMPH on SIP may have been due to AMPH-induced behavioral responses. In fact, AMPH produces dramatic psychomotor responses; a low dose of AMPH enhances locomotor activity whereas high-dose conditions leads to a variety of smaller repetitive movements called “stereotypy” (Shen et al., 2001). “Stereotypy” presents itself in rats through behaviors such as sniffing, repetitive head and forelimb movements, and rearing (Shen et al., 2001). These AMPH-induced behavioral patterns may be related with the suppressing effects of AMPH on water consumption as licking competes with locomotor or other AMPH-induced activities (Didriksen & Christensen, 1994; Liu et al., 2005).

Furthermore, the influence of ovarian steroid hormones on the development of adjunctive characteristics of self-injurious and stereotypic behaviors has been investigated (Becker, Molenda, & Hummer, 2001). They found the different profiles of sensitization for a group of stereotypic behaviors; ovariectomized females treated with estradiol benzoate showed more exaggerated stereotypic behaviors compared to the groups of ovariectomized females, castrated males, and SHAM castrated intact males. Namely, estrogen has a rapid, direct effect on the dorsal striatum which plays an important role in releasing dopamine, wherefore it affects the behavioral response in reaction to psychomotor stimulants.

Thus, it is worthwhile to consider the significant effect of stress on the risk of addiction, because neurobiological links exist between stress and drug abuse (Sinha, 2008). Most importantly, females seem to have sex-specific vulnerabilities which may be linked to stress, underlying drug abuse (Becker et al., 2007). However, the majority of the studies under the SIP paradigm concentrated on male rats. Also, a very small number of relevant studies were published to understand sex differences. Therefore, the aim of this work is to examine sex differences at the behavioral level of AMPH-induced psychomotor responses in the context of stressful situations induced by SIP.

RATIONALE

In summary, this study had two primary purposes. The first purpose was to examine whether injecting d-amphetamine in both male and female polydipsic rats provokes any changes on water consumption, by administering a range of doses of d-amphetamine in those rats. It was expected that both of male and female polydipsic rats would show reduced amount of water intake in a dose-dependent manner.

The second purpose of the current study was to study both of male and female rats concurrently in line with SIP to investigate sex differences, and this research would be the first study reporting sex differences in the current literature of SIP. Regarding sex differences in these experiments, firstly we looked at if there is any significant sex differences between male and female rats on the development and maintenance of SIP as well as among female rats on the different estrous cycle. Next, we determined if there was any significant differences not only between male and female rats on the polydipsic behavior after the administration of AMPH, but also among the distinct estrous cycle of phases of rats.

METHODS

Animals

Training began with a total of 24 Sprague-Dawley (12 of male and 12 female, respectively) rats; however, two of male rats and three female rats (total five) were excluded from this experiment since they failed to develop SIP. As a result, 10 male and 9 female rats continue to be used throughout the experiment. Each animal was individually housed in a single standard shoe-box cage with basic provisions. Both temperature and humidity were controlled with a 12 hour light/dark cycle (lights-on from 05:00 to 17:00). Animals were reduced to 90% of their free-feeding body weights and held at that level for the duration of the experiment by limiting access to food to a single feeding per day. Water, on the other hand, was freely available for each rat. Their daily home cage water intake was recorded every 24 hours.

Drugs

D-amphetamine was obtained from Sigma Aldrich, Inc. (St. Louis, MO). *D*-amphetamine was dissolved in physiological saline (0.9% NaCl) in concentrations of 0.25, 0.5, and 1.0 mg/kg (Liu et al., 2005). Doses refer to the salt form of the drug. Injections were given intraperitoneally 30 min before the session.

Apparatus

Standard rat operant chambers (ENV-008-VP, MED Associates, St. Albans, VT) were used in this study. The interior dimensions of the chambers were 30 x 24 x 29 cm. Fans were set up in the cabinets to mask noise and provide ventilation. A food hopper tray was centered on the stainless steel wall of the chamber, and a retractable lever was located beneath the food hopper.

A water bottle was positioned outside the operant chamber. The hole providing access to a water bottle sipper tube was positioned to the right of the food pellet tray. A lickometer was attached to the metal sipper tube and the grid floor of the chamber. Lever presses and contacts with the sipper tube were recorded by Med PC version, IV for Windows (Med-Associates Inc.).

The chambers are opened by two swinging doors and a camera (Swann pro-series HD 720p) was mounted to the top-left section of the left-side door for recording each session. The test of locomotor activity was carried out in the aforementioned chambers and was quantified using the automated activity video tracking systems (Ethovision XT, Noldus Information Technology, Wageningen, The Netherlands).

Experimental Procedure

Behavioral Measurement

Schedule-induced polydipsia

For the acquisition of SIP, rats were first trained with a fixed time (FT) 60-s schedule of reinforcement. That is, a total of 30 food pellet (single 45-mg food pellet) was delivered to the rats every 60 seconds independently of any response. The animals were shown to have all food pellet reinforcements placed on the pellet magazine over this training session.

Following the preliminary food magazine training, the schedule of FT 60 reinforcement was transitioned to a schedule of a fixed ratio of one (FR-1) reinforcement. Under this FR-1 schedule of reinforcement, the rats were required to earn a total of 30 food pellets throughout a 30 min session by pressing a lever. An FR1 training session ended after 30 reinforcers had been earned or an hour had elapsed.

After the bar-pressing behavior was trained (an FR1 training session resulting in 30 reinforcers within a half hour), a time interval was introduced among the delivery of food

reinforcements; the FR-1 schedule of reinforcement was shifted to a fixed interval (FI) 2.5-second schedule of reinforcement. Then, the 2.5-second interval sequentially increased to 5, 10, 20, 40, and finally 60-second intervals. When the rats reliably maintained FI60" performance (at least 5 reinforcers in a 30 min session), the water bottles were placed in the operant chambers as a preparation for the onset of SIP training session.

Throughout a 30 min SIP training session, the following measures were recorded for each rat in each session: 1) the number of pellets earned, 2) the number of licks on the water spout, and 3) the volume of water consumed. The SIP training sessions (30 min/day) continued for approximately two months. If a rat consumed more than at least 7 ml water, the rat was considered to establish SIP. The rat was considered to achieve stable drinking behavior when his or her value of water consumption was included in the range of ±15% of the amount of water intake for five consecutive days.

Once each rat reached stable levels of polydipsic behavior, a mass feed test session was conducted. The session consisted of having 30 food pellets available in the food tray prior to the start of the session. The lever was retracted throughout the session while the water bottle was still located at the right of the food pellet tray; i.e. the rat was given access to water during the session. This was to demonstrate that the rats were not physically thirsty, and furthermore, that the periodic delivery of reinforcement induced polydipsic behavior in the rats. This mass-feeding session continued for three consecutive days, and the volume of water consumed during each session was measured. Substantial reduction in water consumption was observed under the mass-feeding sessions compared to SIP training sessions.

Amphetamine Testing

After completion of three consecutive mass-feeding sessions, the rats resumed SIP training sessions. When the rats returned to stable levels of polydipsic behavior, established SIP rats began AMPH drug testing. At the start of drug testing, saline was first administered to habituate the rats to intraperitoneal injections. Then, the rats were tested with doses of AMPH, beginning with AMPH vehicle (saline), followed by 0.25, 0.5, 1.0 mg/kg doses in an incremental order. After each AMPH testing day, each rat had the following day off as a washout period. A training session was conducted on the next day, the rats were then monitored for the display of SIP behavior. If SIP behavior was present, the rat would then be administered the next dosage level. If SIP behavior was absent, then SIP training regiments would resume until SIP was re-established.

AMPH-induced Locomotion

AMPH-induced locomotion activities were recorded for 30 min during the SIP test session. Locomotor activity was recorded as the distance of path length in the front half of the chamber and back half of the chamber.

AMPH-induced Stereotypic Behavior

The frequencies of stereotypic behaviors occurring over this 30-min session was counted by an undergraduate assistant. Each 30 min session was divided into six 5-min intervals, and stereotypic behaviors were counted based on the scale provided by Ellinwood and Balster (1974). The scale of stereotypies was revealed as: Score 1, lying down, eyes closed (i.e., asleep). Score 2, lying down, eyes open (i.e., inactive), Score 3, normal grooming or chewing cage litter (i.e., regional activities). Score 4, moving about the cage, sniffing, rearing (i.e., alert and active). Score 5, running movement (i.e., hyperactive). Score 6, repetitive exploration of the cage at a

normal level of activity (i.e., slow patterned behavior). Score 7, repetitive exploration of the cage with hyperactivity or biting attacks (i.e., fast patterned behavior). Score 8, remaining in the same place in the cage with fast repetitive head and/or foreleg movement (i.e., restricted behavior). Score 9, backing up, jumping, seizures, abnormally maintained postures, and dyskinetic movements (i.e., dyskinetic-reactive behavior). Only the stereotypies occurring in the facultative phase of the AMPH-induced SIP sessions were taken into account with counting.

Vaginal Cytology

The vaginal cytology procedure follow the methods reported by Cora et al (2015). Microscopic examinations of vaginal smears were performed to determine the estrous cycle phases of female rats. Cell collection occurred five to 10 minutes after each female rat completed her session.

Vaginal fluid was collected by inserting a 200 ul of pipette tip filled with 0.2 mL of normal saline into the vaginal orifice at a depth of approximately 5-10 mm. The saline was gently pumped into the vagina and pulled in and out approximately two or three times until the vaginal fluid was cloudy. Then, the vaginal secretion was transferred to a microscope slide. When the vaginal fluid was dried out, the dry-fixed slide was stained with crystal violet and then examined under a microscope.

The estrous cycle in female rats is generally divided into the four stages – proestrus, estrus, metestrus, and diestrus. The length of the estrous cycle is average 4-5 days, but it may be shorter or longer than the average length in individual rodent. The average duration of each stage of the estrous is as following; proestrus (14 hr), estrus (24 – 48 hr), metestrus (8 hr), and diestrus (48 – 72 hr). There are four cell types identified during the estrous cycle; small nucleated

epithelial cells, large nucleated epithelial cells, anucleated keratinized epithelial cells, and neutrophils.

Each stage of the estrous cycle in the female rats was determined by the described four cell types of the estrous cycle present or absent on the slide (Cora et al., 2015). The density of cell types is also an indicator to identify each stage of the estrous cycle in female rats (for more details see Cora et al., 2015). The stage of proestrus is characterized by the predominant presence of small nucleated epithelial cells in the cell population. Low numbers of neutrophils may be observed during the transitional period of from diestrus to proestrus. On the other hand, low numbers of anucleated keratinized cells may emerges as the cycle proceeds toward estrus, and this cell type predominantly outnumbered by other cell types and predominated in estrus. In the late estrus of rats, the appearance of nucleated epithelial cells returns back.

Metestrus is the stage where a combination of epithelial cells and neutrophils appears with very high cellularity. As female rats transitioned from metestrus to diestrus, the number of anucleated keratinized epithelial cells is substantially decreased. Meanwhile, a substantial increase of neutrophils is observed with moderate or low cellularity as diestrus progresses. In late diestrus, the epithelial cells may become more round in small clumps, indicating proestrus approaches and it may be observed the next day. Figure 1 and 2 represent the vaginal smears of each estrous cycle.

Data Analysis

This study used the following dependent variables: total number of lever presses, total number of reinforcers, total water consumption, total amount of licks, total distance traveled, and total frequencies of AMPH-induced stereotypy. Values reported as percentage of baseline were

derived by dividing the raw score by the mean of the previous training session and multiplying the result by 100.

In order to measure the temporal locus of lever pressing and drinking between reinforcements during interreinforcement intervals, an index of curvature was calculated. Fixed intervals (i.e., one minute per session in this experiment) were divided into five 12-sec periods which corresponded to individual bins, and then the number of lever presses and licks which preceded each pellet delivery were collected in each bin. The mean for each bin for each animal and session was calculated to determine the curvature for the frequency of licks occurring between each food pellet. The formula used for the calculation was:

$$IC = \frac{4(Bin1 + Bin2 + Bin3 + Bin4 + Bin5) - 2(4 \cdot Bin1 + 3 \cdot Bin2 + 2 \cdot Bin3 + Bin4)}{5(Bin1 + Bin2 + Bin3 + Bin4 + Bin5)}$$

(Fry, Kelleher, & Cook, 1960).

An independent samples t-test was used to compare the number of sessions to meet the SIP training criteria between the male and female rats. A paired samples t test was to assess differences in mass feeding session water consumption and total licks from baseline. A two-way repeated analysis of variance (ANOVA) test, using sex as the between-subjects factor and the AMPH dose as a within-subjects factor, was used for analyzing operant performance, locomotion, and stereotypy. Whenever appropriate, post hoc comparisons were made using Bonferroni correction to reveal the difference between group pairs of interest.

Finally, an analysis was conducted on training sessions in females that had met the training criteria in order to compare results between the proestrous and diestrous phases using a dependent samples t test for number of lever presses, reinforcers, amount of water consumed,

and distance traveled. A paired samples t test was used to assess differences in mass feeding session water consumption, total licks, and the index of curvature from the baseline. All comparisons were based on two-tailed probabilities and the criterion for statistical significance was $P < 0.05$. All analyses were conducted using SPSS for Windows (v. 25).

RESULTS

Acquisition and Development of SIP

Of the initial 24 animals obtained for this study, ten males and 9 females, respectively, began consuming water from the bottles after 8.9 (+/- SEM 1.9) training sessions. To meet the training criteria for SIP (±15% of the amount of water intake for five consecutive day), a mean of 24 days (+/- SEM = 2.55) was required. Between the males and females, male rats (M = 18, SEM = 2.15) met the criteria in significantly fewer sessions than the female rats (M = 28, SEM = 3.97), $t(17) = -2.28$, $p = 0.036$ (Figure 3). However, a significant difference was not found in the total water consumption during the SIP training sessions between males (M = 9.62, SEM = .83) and females (M = 8.12, SEM = 1.05); $t(17) = 1.13$, $p = .275$.

The Effects of Estrous Cycle on SIP during Training Sessions

Of the initial 14 animals subjected for SIP training sessions, two female rats only displayed the diestrus stage during a month-and-a-half. One of them ended up being excluded from this experiment since the rat did not acquire SIP at all. The rest of the female rats had average 4-5 days of the estrous cycle, however, it was difficult to include each estrous cycle as the data. Figure 4 represents the total amount of water consumed during 5 consecutive days prior to meet the first criteria of stable level of polydipsia based on their estrus stages.

After an animal met the training criteria for SIP the mass feeding procedure was conducted. Figure 5 presents the percentage of water consumption and the percentage of total licks as compared to the baseline. Mass feeding significantly decreased water consumption when

compared to the baseline, $t(18) = 11.68$, $p < 0.001$. Mass feeding also significantly decreased total licks compared to the baseline, $t(18) = 7.49$, $p < 0.001$.

Behavioral effects of AMPH on Lever Pressing and Reinforcer

Figure 6 represents the percentage of the number of lever pressing made, compared to baseline, during each drug testing session including vehicle. Figure 7 represents the percentage of the number of reinforcers delivered, compared to baseline, during each drug testing session including vehicle.

The results of the two-way repeated measures ANOVA revealed that there was a main effect of sex differences on total lever responses $F(1, 17) = 4.537$, $p = 0.048$. The total number of lever pressing was also statistically different for the main effect of dose, $F(3, 51) = 2.905$, $p = 0.044$. There was also not a significant interaction between sex and dose on total lever responses, $F(3, 51) = 0.787$, $p = 0.507$.

Because descriptive statistics revealed that rats' mean total lever pressing responses were higher when administered AMPH 1.0 mg/kg (M = 129.90, SEM = 14.27) compared to vehicle (M = 107.30, SEM = 4.95), a further statistical analysis was conducted using only 1.0 mg/kg and vehicle in a combined group of male and female rats using a dependent samples t test. However, a statistically significant effect was not found, $t(18) = -1.459$, $p = 0.162$.

The results of the two-way repeated measures ANOVA revealed that there was significant sex differences between the males and females on the number of reinforcers earned, $F(1, 17) = 5.96$, $p = 0.026$. A statistically significant effect was also neither found for drug ($F[3, 51] = 1.29$, $p = 0.289$, nor for an interaction, $F(3, 51) = 1.29$, $p = 0.289$.

Behavioral effects of AMPH on Licks, Water Intake, and Index of Curvature

Figure 8 represents the percentage of the total number of licks registered, compared to baseline, during each drug testing session including vehicle. Figure 9 represents the percentage of total water consumed, compared to baseline, during each drug testing session including vehicle. Figure 10 represents the percentage of index of curvature, compared to baseline, during each drug testing session including vehicle.

The total licks made during the testing sessions were analyzed with a two-way repeated measures ANOVA (dose X Sex). A statistically significant main effect of sex was not detected, $F(1, 17) = 1.846$, $p = 0.192$. On the contrary, a statistically significant main effect of AMPH was detected, $F(3, 51) = 2.897$, $p = 0.044$. However, a pairwise comparisons with Bonferroni correction post hoc test did not identify statistically significant differences between groups (see Table 1). There was not an interaction effect between dose and sex on the amount of licks registered during the testing sessions, $F(3, 51) = 0.150$, $p = 0.929$.

A two-way repeated measures ANOVA was also conducted to compare the male and female differences in total water consumption during the SIP testing sessions with across doses of AMPH. The statistical analysis did not indicate significant differences in the difference in total water intake consumed for sex, $F(1, 17) = 0.563$, $p = 0.463$. Although there was not a significant main effect for sex, there was a significant main effect for AMPH, $F(3, 51) = 2.949$, $p = 0.041$. However, significant differences were not shown from the post hoc tests (see Table 2). There was also not a significant interaction with sex and dose, $F(3, 51) = 0.441$, $p = 0.725$.

A two-way repeated measures ANOVA was also ran to compare the male and female differences in the index of curvature during the SIP testing sessions with across dose of AMPH. The statistical analysis did not find significant differences in the difference in the index of

curvature for sex, F(1, 17) = 0.373, p = 0.550. A statistically significant effect was also neither found for drug (F[3, 51] = 2.47, p = 0.072, nor for an interaction, F(3, 51) = 1.95, p = 0.134.

AMPH-induced Locomotion

Figure 11 represents the total distance traveled during each drug testing session including vehicle. The results showed the main effect of sex was non-significant, F(1, 17) = 0.009, p = 0.928. However, the effect of AMPH was dose-dependently significant on the total distance in SIP, F(3, 51) = 4.907, p = 0.005. The pairwise comparison of within-subjects (dose) showed that injection of AMPH 0.25 mg/kg (p = 0.045) and AMPH 0.5 mg/kg (p = .009) significantly increased the total walking distance of locomotion more so than vehicle injection. However, AMPH at the largest dose of 1.0 mg/kg did not have significant effect (p = 0.108). There was not a significant interaction between the effects of dose and sex on AMPH-induced locomotor activity, F(1, 17) = 0.443, p =0.723.

AMPH-induced Stereotypic Behavior

Figure 12 represents the total stereotypic behavior occurring during each drug testing session including vehicle. The frequencies of stereotypy activity occurring in the facultative period over the testing sessions had been recorded. Stereotypy activity were counted by every 5-min during the 30-min period after injection of saline or drugs (AMPH; 0.25, 0.5, or 1.0 mg/kg). A two way repeated ANOVA was employed for the statistical analysis. The results showed the main effect of sex was non-significant, F(1, 17) = 0.012, p = 0.915. The main effect of AMPH on the total distance in was also not found, F(3, 51) = 1.096, p = 0.359. There was not a significant interaction between the effects of dose and sex on AMPH-induced stereotypic behavior, F(3, 51) = 0.429, p =0.733.

DISCUSSION

The ultimate objective of this experiment was to examine significant sex differences in the amount of water consumed when the animals were administered AMPH. This study also aimed to examine the effects of the estrous cycle in female rats to determine if behaviors may change when estradiol levels are naturally high or low and how these levels interact with psychostimulant drugs. It was hypothesized that a dose-dependent decline would occur in both the male and female rats, resulting from the administration of AMPH as reported by previous studies. It was further hypothesized that the female rats would show higher drop rates in water consumption compared to the baseline, relative to the male rats, in consequence of the female rats' estrogen modulation. AMPH-induced abnormal movements as well as schedule-controlled responses (i.e., lever pressing) were also examined.

Acquisition and Development of SIP behavior

Sex differences in the appearance of SIP were investigated during the acquisition and development period of SIP. It was assessed whether there were any sex differences in the behavioral pattern of polydipsia during the generation of SIP. Interestingly, the female rats needed significantly more sessions to develop stable patterns of polydipsia compared to the males. Although sex was discovered as a significant effect across the total sessions taken for the development of stable polydipsia, the total amount of water consumed between males and females was not significantly different. These observations have two implications.

First of all, these findings may be interpreted as the female rats being 'slower' to develop SIP behavior, but once they acquire SIP behavior, both the male and female rats exhibit identical

manifestations of SIP as excessive, repetitive drinking behavior. It is important to note that "slower at developing SIP behavior" does not imply these female rats are slower at learning, rather that schedule-induced drinking is not learned through the response-reinforcer contingency employed for the reinforcing conditions. Alternatively, schedule-induced drinking is induced as a function of fixed interval length.

Previous studies have reported that anxiety-prone female rats showed lower propensity for exploration in the open arms and a higher level of anxiety compared to male rats (Donner & Lowry, 2013; Palanza, 2001). It has also been implicated that estrous cycle influences the state of anxiety and anxiety-related responses of female rats (Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001). With this information altogether, the female rats' tendency to develop SIP behavior at a slower pace may suggest higher levels of anxiety accompanied with slow psychological processing in a SIP task. That being said, assuming that the 'coping hypothesis' is true for the cause of SIP, drinking behavior was adopted by the female rats as a coping mechanism for anxiety which was induced by the experimental condition (i.e., an intermittent schedule of reinforcement), and their higher level of anxiety compared to the male rats is manifested by the slow-paced development of SIP behavior. This assumption needs to be addressed in future studies.

There was a chance that a possible procedural error occurred in the data collection procedures. When water bottles were placed outside the chambers during each SIP training sessions, these water bottles were hung upside down so that the sipper tube could be inserted close to each rat's height. During the experiment, the possibility for leakage was prevalent as the caps in which the sippers were attached may have come loose. The possible leakage may have

conflicted with valid and reliable data collection for the total water consumption during the earlier SIP training sessions.

The effects of the estrous cycle during the development of stable polydipsia could not be investigated. This was because some short stages of the estrous cycle (i.e., proestrus) were frequently missed during vaginal smear collections. Although the effects of hormonal fluctuations during the development of stable levels of polydipsia could not be examined (explained in the discussion section), the mean (simple average) amount of water consumed for the five consecutive days required to meet the first criteria for a stable level of polydipsia was calculated by the estrous cycle.

The results were as follows: the female rats consumed 14.13 mL (SEM = 2.15) of water during their proestrus, 13.7 mL during their estrus (SEM = 1.55), and 12.17 mL during their diestrus stages (SEM = 1.13). In spite of not having enough data to further analyze, these findings would seem to suggest that there might have been an interaction occurring between ovarian hormones and psychological stress induced by a SIP task. According to a review of the literature, female rats' normal daily fluid intake appears to be influenced by ovarian estrogen levels (Tarttelin & Gorski, 1971). In this study, daily water intake by the female rats in their home-cages was measured for several months while tracking each rats' estrous cycle as a moderating variable that may affect drinking behavior. The results showed a substantial decrease during the estrus stage of the estrous cycle where the elevated estradiol levels during proestrus returned to the baseline as ovulation occurred.

In addition, the modulating properties of estrogen to behavioral responses during stress-tests have been reported (Ter Horst, Wichmann, Gerrits, Westenbroek, & Lin, 2009). That being said, it is possible that the influence of ovarian steroid hormones on stress coping may have

engaged in polydipsic behavior during the rat SIP assay. Further studies will need to be undertaken to determine whether the concentration of estrogen is indeed implicated in the compulsive water intake in a SIP procedure as a coping mechanism with stress.

During the SIP training sessions, the animals showed negative index of curvature reflecting drinking occurring at the beginning of an interval after the pellet was delivered and was followed by a short pause of 1-min before the next pellet. Contrasting the negative index of curvature reflected in drinking as mentioned above, positive values of index of curvature were calculated for lever pressing in the animals. Thus meaning they pressed the lever slowly at first, but with increasing frequency as the end of the interval approached.

The influence of AMPH on changes in operant performance, SIP, and psychomotor movements

Previous studies have reported that AMPH led to a reduction in water intake and licks during SIP (Didriksen & Christensen, 1994; Liu et al., 2005; Segal, Oden, & Deadwyler, 1965). These earlier findings were replicated in this study: total water consumption was dose-dependently decreased. The highest dose of AMPH induced the largest decline in water consumption compared to the baseline, potentiating its effect on schedule-induced drinking behavior. AMPH also dose-dependently decreased the total number of licks made during drug testing sessions. A significant sex difference on water consumption was not found, and this may support the notion discussed above that polydipsic behaviors which had been developed may not be influenced by sex during the maintenance of SIP.

According to previous findings**,** the behavioral activating effects of AMPH interfere with schedule-induced polydipsic behavior. In fact, greater behavioral activation occurs in AMPH-induced rats, and this AMPH-induced behavioral activation may present itself in different ways

such as increased vigilance, greater presence of stereotypic behavior, and heightened locomotor activity (Koelega, 1993). More specifically, AMPH induces a biphasic response in animals, predominantly exhibiting increased locomotor activity at a lower dose and becomes primarily displaced by stereotypic behavior at higher doses of AMPH (Yates, Meij, Sullivan, Richtand, & Yu, 2007). The animals in this experiment also demonstrated increased locomotor hyperactivity, and repetitive, purposeless stereotypic movements (This will be discussed in detail below).

The effects of gonadal hormones have also been reported in AMPH-induced behavioral activating effects. It has been implicated that the level of estradiol potentiates behavioral sensitization to AMPH, which leads to sex differences in the manifestation of AMPH-induced behavioral sensitization (Camp & Robinson, 1988). Thus, it was observed that female rats exhibited greater and more rapid sensitization of locomotor activity and stereotypic behavior than male rats. Contrary to the findings of previous studies, we did not find significant sex differences on the AMPH-induced stereotypic behavior. This will be discussed later in this section.

It has been suggested that the AMPH-induced behavioral sensitization (i.e., increased locomotor activity and enhanced stereotypic responses) might have a suppressing impact on schedule-induced polydipsic behavior (Liu et al., 2005). Additionally, given the stress-reducing properties of polydipsic behavior, the reduced licks and water intake in rats could be interpreted as behavioral consequences of the reinforcing effects of AMPH under stress exposure. In line with previous studies, a similar pattern of results was obtained in this present study; the administration of AMPH dose-dependently produced sensitized increases in locomotion. Further analysis (Bonferroni test) even revealed that the lower doses of AMPH (0.25 and 0.5 mg/kg)

increased locomotor activity compared to the baseline. The highest dose of AMPH (1.0 mg/kg) in this experiment did not increase locomotor activation in the animals.

Interestingly, escalating doses of AMPH in this experiment did not elicit a significant difference in stereotypic behavior in the animals. One possible explanation for failing to replicate AMPH-induced stereotypy demonstrated in the previous findings may have been due to the range of doses tested in this study. The dosage range in this experiment may not have approached the 'high' threshold conducted by other experiments. The highest doses tested in this current research was 1.0 mg/kg, but much higher doses of AMPH had been used in previous findings which confirmed to induce hyperactivity and stereotypy (Antoniou, Kafetzopoulos, Papadopoulou-Daifoti, Hyphantis, & Marselos, 1998). Overall, these findings are in accordance with findings reported by previous studies.

Some writers reported that the excessive amount of drinking behavior adopted as a way to cope with stress may have been transitioned to nose poking behavior in rats (Liu et al., 2005). In their studies, licks and water intake were diminished whereas nose-poking was augmented at the same time. That being said, injection of AMPH might have improved the motivational states of rats, and consequently the animals increased the amount of nose-poking which reflected an inner drive toward the reinforcement (i.e., food pellets) (Liu et al., 2005).

In this experiment, a similar conclusion was reached by the similar pattern of behavior in the animals. The results demonstrated AMPH injections produced dose-dependent increases in the schedule-controlled lever pressing in both the male and female rats. It may suggest motivational properties of rewarding behavior in AMPH administrations. Furthermore, a sex difference was found in the number of lever presses. The female rats increased lever pressing to

a higher degree than the male rats. This may reflect on the vulnerability of female rats in the reinforcing effects of psychostimulants.

With respect to the magnitude of behavioral sensitization, the female rats did not exhibit greater AMPH-induced behavioral sensitization of neither locomotor activity, nor stereotypic behavior. As discussed above, stereotypic sensitization may not have been induced due to the lower doses of AMPH tested in this experiment. However, it remains unclear why AMPH injections did not provoke greater excessive motor activities in the female rats compared to males. It is speculated that this might have been due to the lack of proestrus stages during the drug testing sessions. If the female rats had been given AMPH during their proestrus stages when the level of estradiol is at peak, they may have shown greater psychomotor activities since estradiol enhances sensitization.

This paper intended to contribute to the existing literature on SIP. The majority of the studies under the SIP paradigm concentrated on male rats. The current study aimed to include not only male rats, but also their female counterparts. The present study confirmed previous findings about behavioral effects of AMPH during a SIP task. Our results cast a new light that female rats may display a different magnitude or pattern of behavior in response to AMPH; however, firm conclusions concerning sex differences in a SIP task cannot be made due to a lack of evidence.

Limitations

As noted above, there were several limitations to the present study. The biggest limitation of this current research was not having enough data collected during proestrus stages. Accordingly, it was difficult to run statistical analysis for the effects of the estrous cycle on the development and maintenance of SIP behavior, and further on AMPH-induced behavioral

performance. This may have resulted from the late schedule of vaginal samples collected during the evening, after 5 p.m., when the lights turned off.

To expand in detail, two of the female rats displayed persistent diestrus stages during the first month-and-a-half since their SIP training session had begun. In this experiment, the first couple of weeks were critical for data collection because this time period was when the majority of the animals started developing their SIP behavior. In an effort to minimize the incidences of missed stages, especially the proestrus stage, vaginal fluid collection/smear sample taking was shifted from the evening to early afternoon. With the performance of vaginal smear sample taking being moved to earlier in the day, the smears displayed proestrus stages in the female rats more frequently. In particular, one of the female rats who did not seem to have regular cycles started to demonstrate normal phases of an estrous cycle. However, the new timeline still did not overlap with a couple of female rats' proestrus phases. This data scarcity contributed to the largest weakness of the current study.

It is not certain that the other female rat who only showed diestrus stages throughout the SIP training sessions had an impairment of reproductive cycles. However, a number of vaginal cytologies were performed at different parts of the day, including early morning and afternoon (between 10 a.m. to 2 p.m.), and late night (between 8 p.m. to 11 p.m.) in order to capture different estrous cycle phases of this specific rat. The other phases of the estrous cycle were not observed at different parts of a day. This female rat seemed to fail to enter a normal estrous cycle. In addition, this rat ended up being excluded from the drug testing sessions since she did not acquire SIP behavior.

It was also challenging to monitor specific phases of females' reproductive cycles. For example, each stage of the estrous cycle was unequal in length for each individual rodent in this

study, and in-general (Cora et al., 2015). While diestrus has an average length of 48 – 72 hr, proestrus has only 14 hr in rats. There was a possibility that shorter phases of the estrous cycle may elapse when vaginal fluid collection was performed. Additionally, the estrous cycle length in female rats could be inconsistent and influenced upon by a number of variables including both external variables (e.g., dim light) and internal variables (e.g., hormonal fluctuation) (Westwood, 2005). Lastly, it was also difficult to identify the transitioning period from one stage to another. Taken together, it was difficult to capture the proestrus moments during the dynamic process of the estrous cycle, and the lack of proestrus stages during the drug testing caused a large limitation in this experiment.

In terms of stereotypic behavior, only one novice observer counted AMPH-induced stereotypies, which in turn, might have weakened the accuracy and precision of observations. At least two observers should have been needed to count the frequencies of stereotypic behavior so that stereotypy data was collected in a consistent way. However, it was practically challenging to obtain assistance when most of the students were gone for summer break. Furthermore, although each duration of 30-min session was broken down into six, 5-min intervals to minimalize the observational error of stereotypy, a greater need for more quantitatively standardized methodologies for measuring stereotypic behavior of rats in the operant chamber seemed necessary.

Another limitation in this study involves the issue of doses tested. As previously mentioned, the four doses employed in this study may not have produced an extensive enough range to induce more distinct effects of AMPH. After AMPH administration, the animals exhibited locomotor hyperactivity in a dose-dependent manner. For example, there was a

remarkable increase in locomotor activity at doses of 0.25 mg/kg and 0.5 mg/kg. On the contrary, a dose of 1.0 mg/kg did not produce a pronounced increase in locomotion.

However, the number of stereotypy occurring during each drug testing sessions was not related with increased dosages of AMPH. Given that stereotypy occurs at a higher dose, we concluded that doses greater than 1.0 mg/kg may have been needed in this study to induce more distinct effects of AMPH. Results might have been different if doses higher than 1.0 mg/kg were included in this experiment.

CONCLUSIONS

This study was the first attempt to examine sex differences during a SIP procedure. While this procedure has been used for decades and is reported in hundreds of published studies, very few have actually used female animals and none have explored a direct comparison between male and female animals. Therefore, the aim of this study was to re-examine a well-characterized animal model in male rats to determine if female rats would exhibit a different magnitude or pattern of behavior in response to AMPH. This current study found that the female rats required more training sessions to develop SIP behavior. In addition, the female rats earned more reinforcers by working harder on lever pressing. However, this study could not further analyze the effects of the estrous cycle on polydipsic behavior in the SIP model because of experimental limitations. Although this study involved limitations due to methodological problems and a lack of data, this study indicated that sex differences may exist on schedule-induced polydipsia. Therefore, this study expects to provide insight for future studies on how to improve methodological procedures. With enhanced methodological procedures being utilized, future studies may reveal a better understanding of estrous cycle-dependent variations in AMPH-induced behaviors on SIP.

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Tables

Table 1

Two-way Repeated Measures of ANOVA (AMPH X Sex) Pairwise Comparisons on Total Number of Licks.

(I) AMPH	(J) AMPH	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Vehicle	AMPH0.25	18.591	13.973	1.000	-23.104	60.287
	AMPH0.5	8.872	9.655	1.000	-19.939	37.684
	AMPH1.0	36.610	15.372	.175	-9.261	82.481
AMPH0.25	Vehicle	-18.591	13.973	1.000	-60.287	23.104
	AMPH0.5	-9.719	9.926	1.000	-39.337	19.899
	AMPH1.0	18.019	15.921	1.000	-29.490	65.527
AMPH0.5	Vehicle	-8.872	9.655	1.000	-37.684	19.939
	AMPH0.25	9.719	9.926	1.000	-19.899	39.337
	AMPH1.0	27.738	11.937	.197	-7.883	63.358
AMPH1.0	Vehicle	-36.610	15.372	.175	-82.481	9.261
	AMPH0.25	-18.019	15.921	1.000	-65.527	29.490
	AMPH0.5	-27.738	11.937	.197	-63.358	7.883

Table 2

Two-way Repeated Measures of ANOVA (AMPH X Sex) Pairwise Comparisons on Total Water Consumption

(I) AMPH	(J) AMPH	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
Vehicle	AMPH0.25	-2.830	9.798	1.000	-32.068	26.407
	AMPH0.5	3.533	4.630	1.000	-10.283	17.349
	AMPH1.0	23.314	11.877	.397	-12.126	58.755
AMPH0.25	Vehicle	2.830	9.798	1.000	-26.407	32.068
	AMPH0.5	6.363	9.165	1.000	-20.984	33.711
	AMPH1.0	26.145	10.596	.147	-5.474	57.763
AMPH0.5	Vehicle	-3.533	4.630	1.000	-17.349	10.283
	AMPH0.25	-6.363	9.165	1.000	-33.711	20.984
	AMPH1.0	19.781	10.711	.494	-12.182	51.744
AMPH1.0	Vehicle	-23.314	11.877	.397	-58.755	12.126
	AMPH0.25	-26.145	10.596	.147	-57.763	5.474
	AMPH0.5	-19.781	10.711	.494	-51.744	12.182

Table 3

Total Water Consumption in the Female Rats by Their Estrous Cycle during SIP Training Sessions

Estrous Cycle	Mean Water Consumption
Proestrus	14.13
Estrus	13.7
Diestrus	12.17

FIGURES

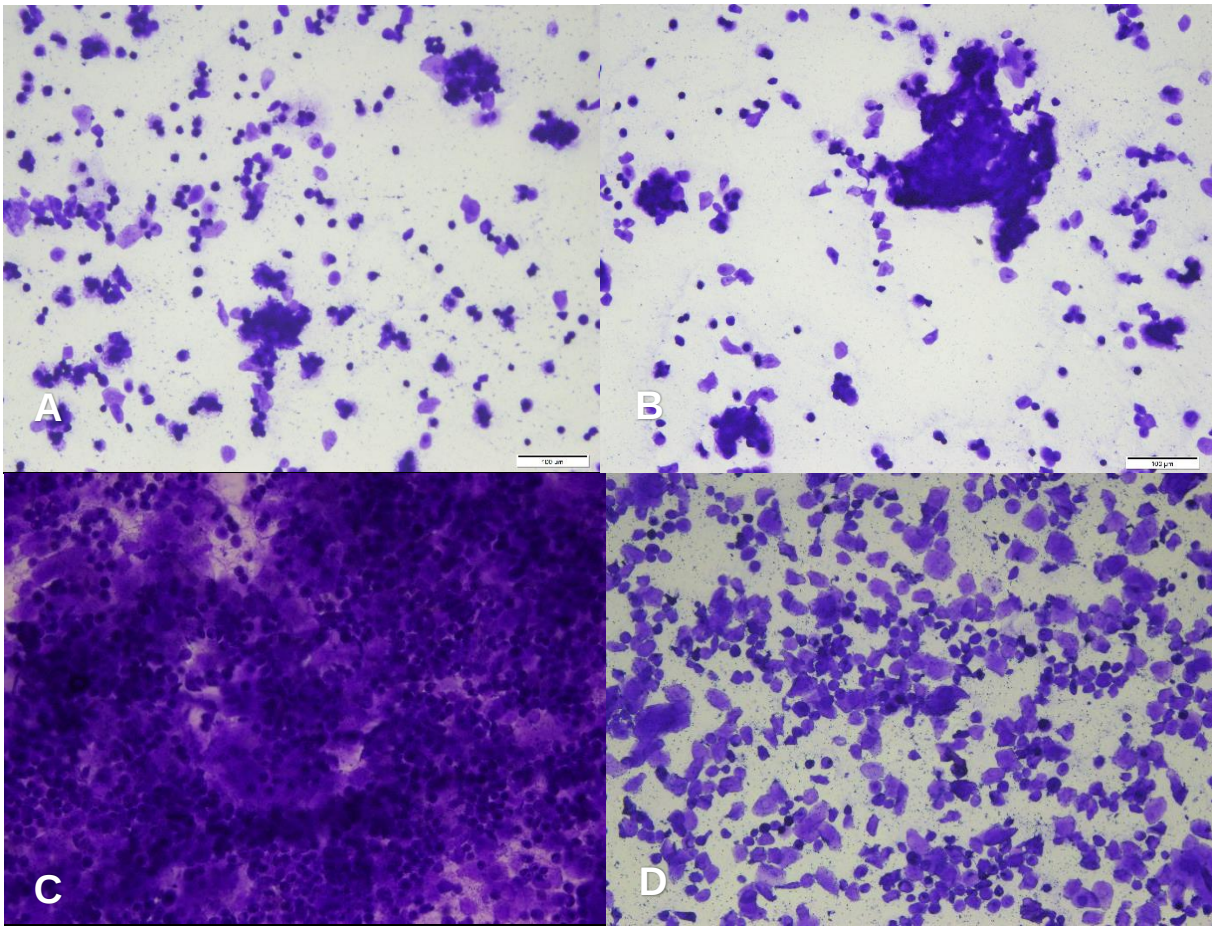


Figure 1. Vaginal smears of proestrus (A – D) in several Sprague-Dawley rats. Plates A and B represent a typical proestrus stage with high numbers of small nucleated epithelial cells found individually and in cohesive clusters. Plate C represents clusters of epithelial cells with a high cellularity during proestrus stage. Plate D represents a proestrus-to-estrus transition. Original objective magnification of 40X.

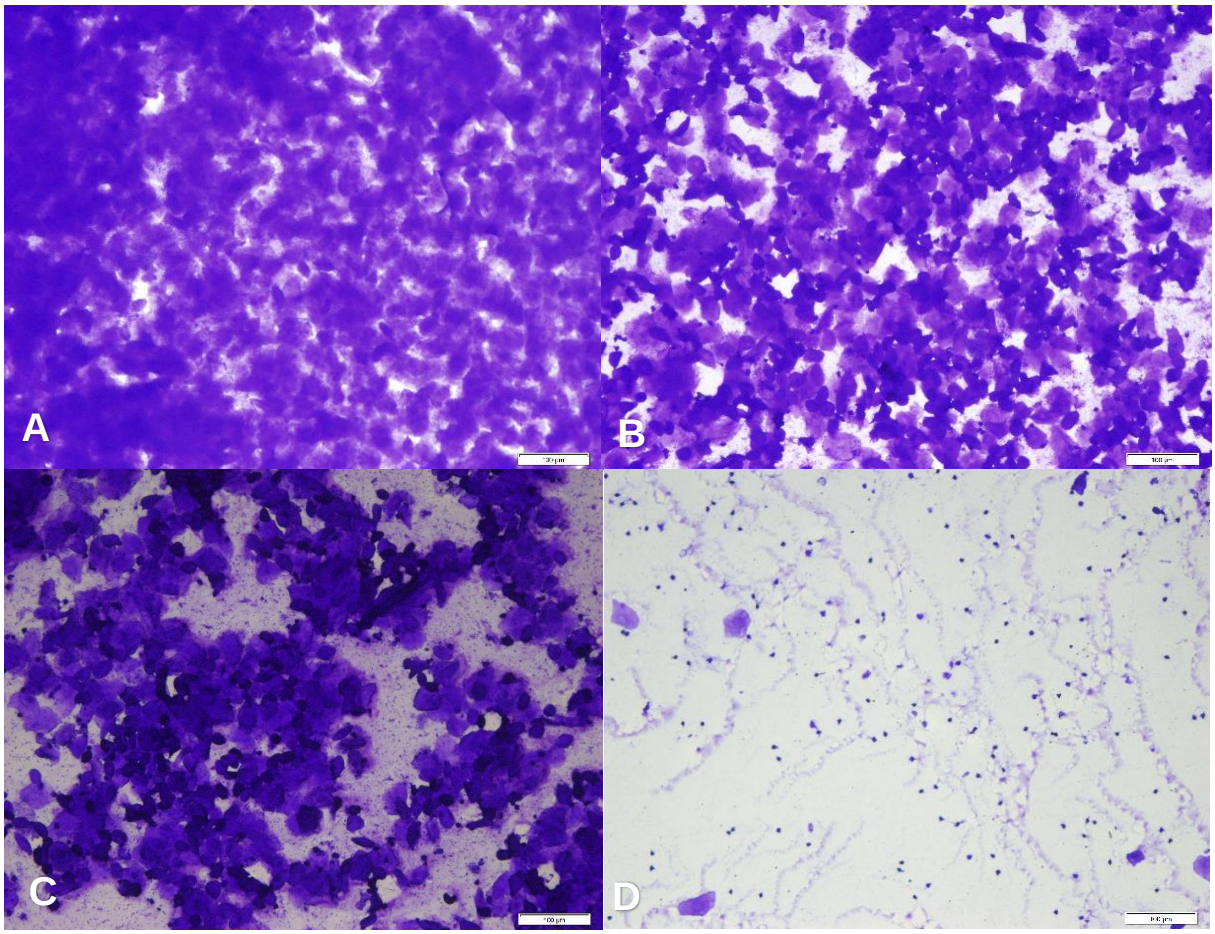


Figure 2. Vaginal smears of estrus (A – B), metestrus (C), and diestrus (D) in several Sprague-Dawley rats. Plates A represents a typical estrus stage with predominant appearance of anucleated keratinized epithelial cells. Plate C represents late estrus characterized by the presence of round-shaped nucleated epithelial cells interspersed among anucleated epithelial cells. Plate C represents metestrus stage with the emergence of neutrophills in the epithelial cells. Plate D represents a typical diestrus stage with predominant neutrophills with a lower celluarity. Original objective magnification of 40X.

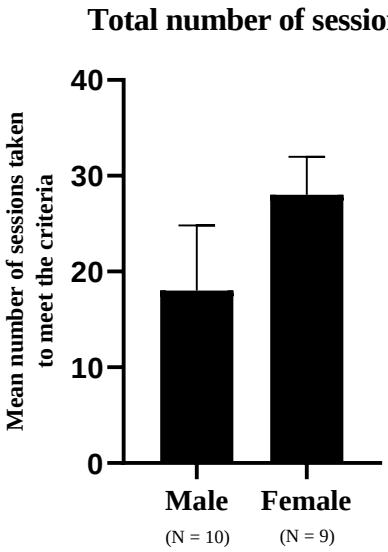


Figure 3 shows mean total sessions between the males and female to meet criteria for the development of stable polydipsia (±15% of the amount of water intake for five consecutive day) in Sprague Dawley rats in the SIP animal model.

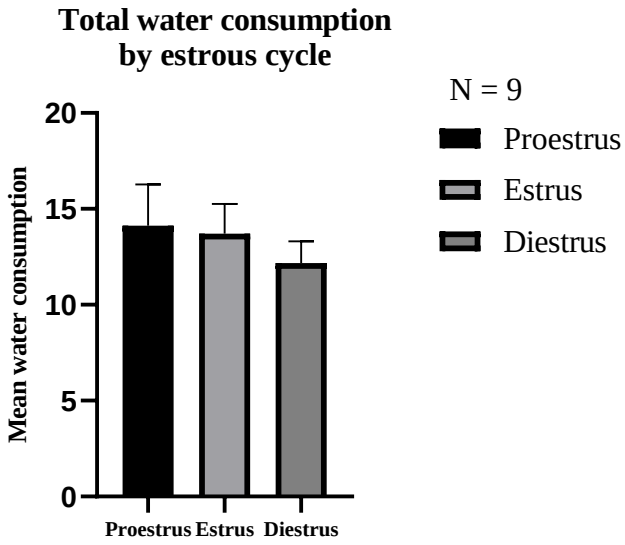
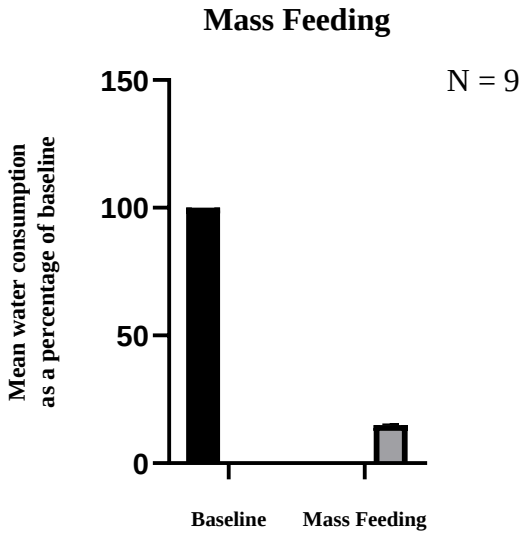


Figure 4 shows total amount of water consumed during the SIP training sessions in the female Sprague Dawley rats by the estrous cycle (Proestrus – Estrus – Diestrus).



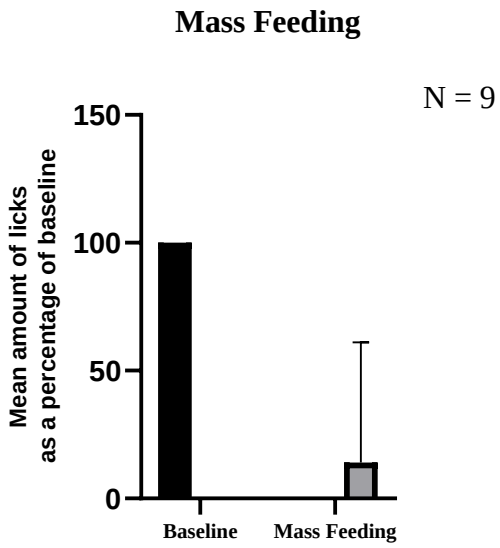


Figure 5 shows water consumption and the amount of licks made (in the form of a percentage of the baseline) during mass feeding administration as a function of sessions in Sprague Dawley rats in the SIP animal model.

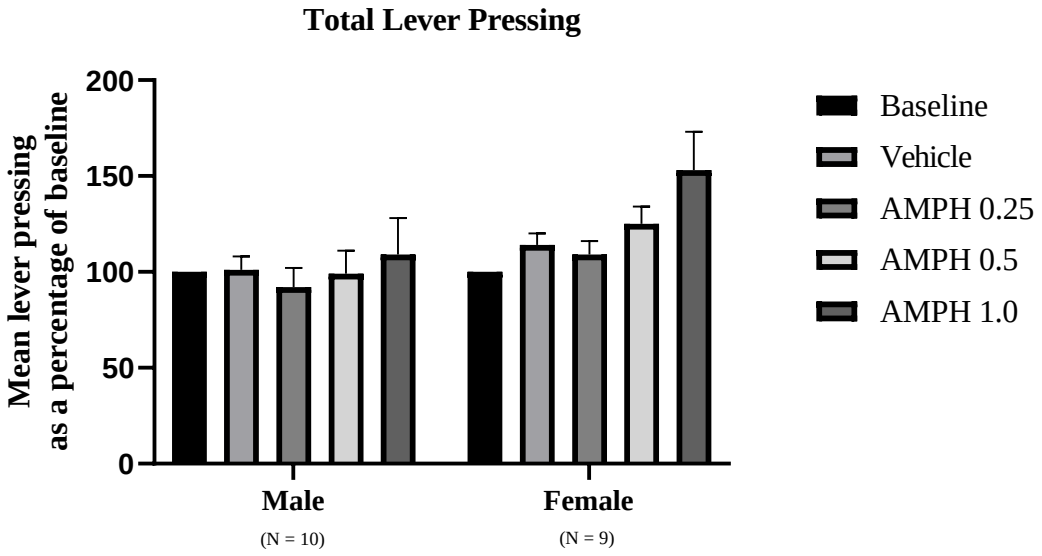


Figure 6 shows the total number of lever pressing made (in the form of a percentage of baseline) during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague Dawley rats in the SIP animal model. Baseline data is the mean of the five prior consecutive sessions to drug testing.

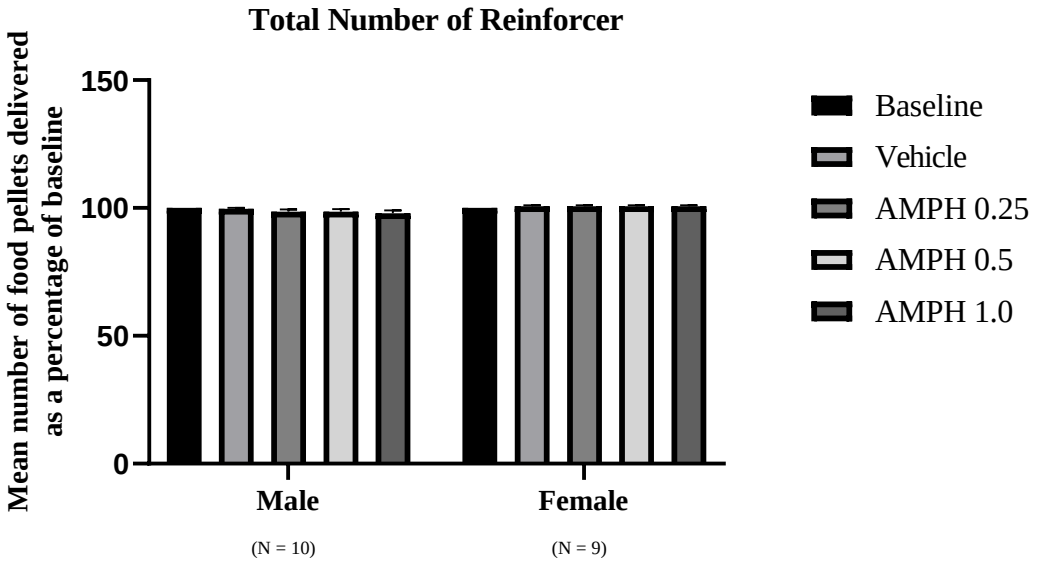


Figure 7 shows the total number of reinforcers earned (in the form of a percentage of baseline) during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague Dawley rats in the SIP animal model. Baseline data is the mean of the five prior consecutive sessions to drug testing.

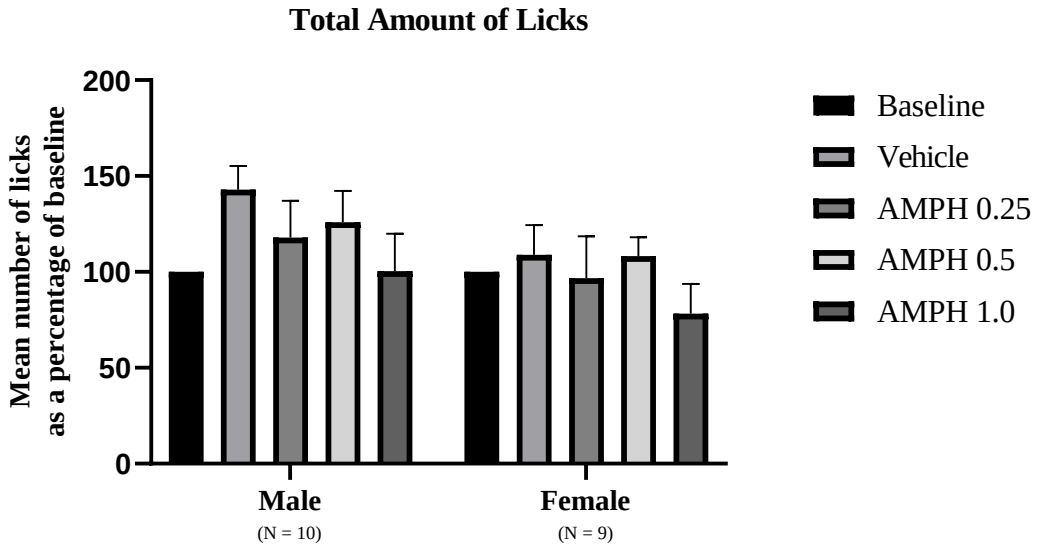


Figure 8 shows the total number of licks registered (in the form of a percentage of baseline) during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague Dawley rats in the SIP animal model. Baseline data is the mean of the five prior consecutive sessions to drug testing.

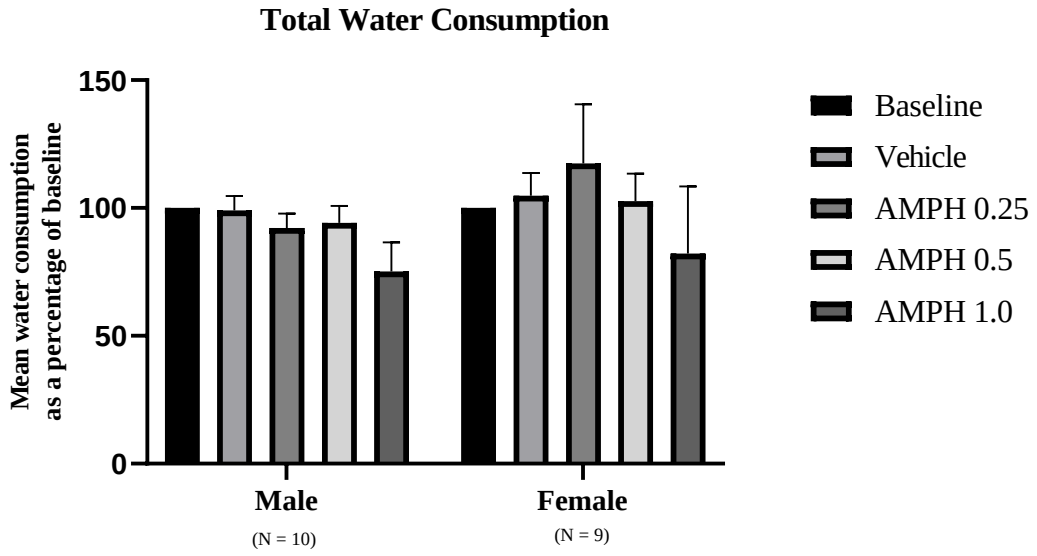


Figure 9 shows the amount of water consumed (in the form of a percentage of baseline) during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague Dawley rats in the SIP animal model. Baseline data is the mean of the five prior consecutive sessions to drug testing.

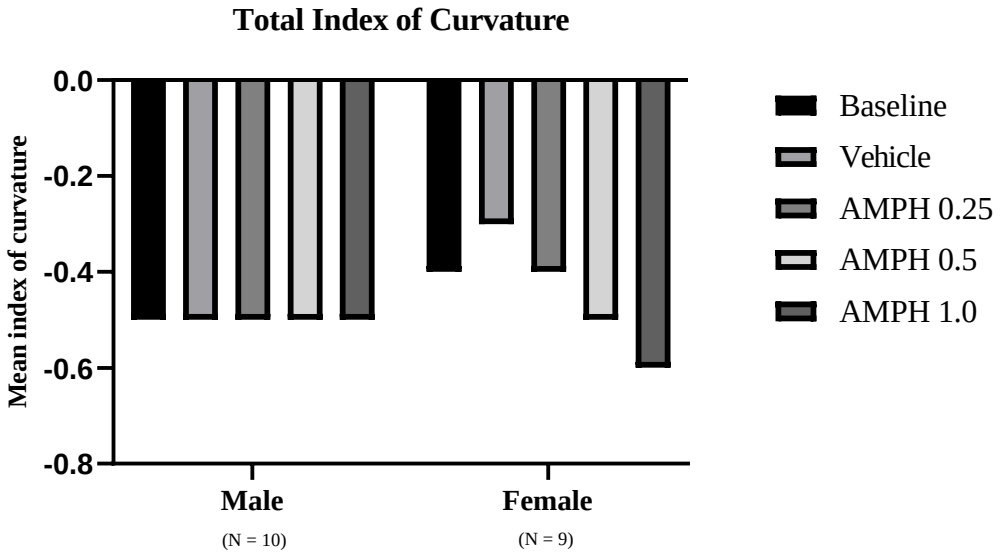


Figure 10 shows the index of curvature during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague-Dawley rats in the schedule-induced polydipsia animal model. Baseline data is the mean of the five prior consecutive sessions to drug testing.

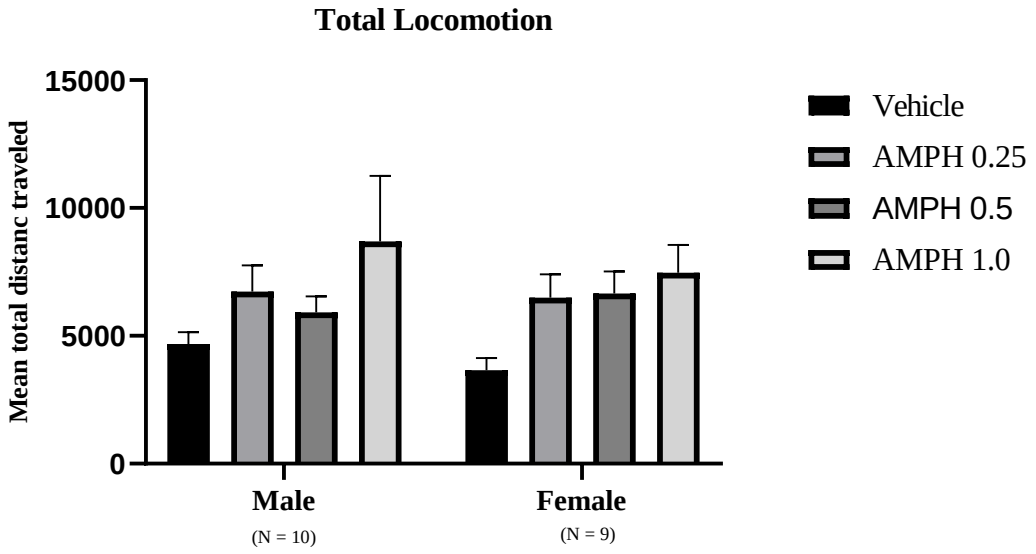


Figure 11 shows total distance traveled during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague-Dawley rats in the schedule-induced polydipsia animal model.

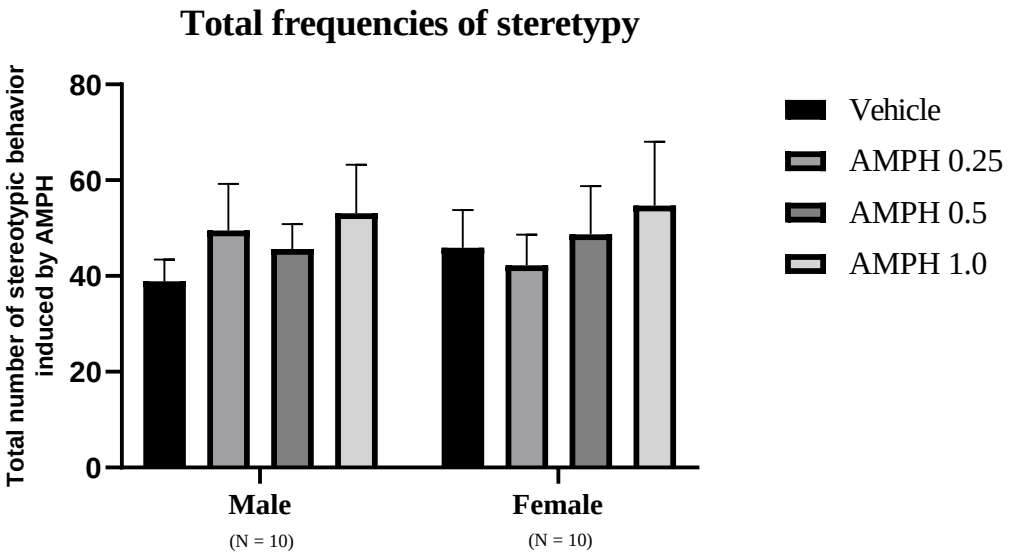


Figure 12 shows total frequencies of stereotypic behavior occurring during the drug testing sessions following vehicle and AMPH doses (0.25, 0.5, and 1.0 mg/kg) as a function of repeated sessions in Sprague-Dawley rats in the schedule-induced polydipsia animal model.

APPENDIX

Institutional Animal Care and Use Committee Approval Form

The approval form from the Institutional Animal Care and Use Committee for use of animal subjects in the present study has been copied and attached.

SIGNATURE PAGE

IACUC #: 349 **PROPOSAL TITLE (From cover page):** Schedule induced polydipsia in male and female rats

X. ACKNOWLEDGEMENT BY PRINCIPAL INVESTIGATOR

I acknowledge responsibility for this project. I have read the Northern Michigan University Principles for the Care and Use of Laboratory Animals and certify that this project will be conducted in compliance with those principles. I assure that I will obtain Institutional Animal Care and Use Committee approval prior to significant changes in the protocol. I assure that this project does not unnecessarily duplicate previous research or instructional projects. I assure that students, staff and faculty on the project are qualified or will be trained to conduct the project in a humane, safe, and scientific manner.

Signature: _____ 01/25/2019

Principal Investigator Date

XI. APPROVAL OF SCIENTIFIC MERIT (to be completed by the Department Head)

Before the project is initiated, it must be reviewed and approved on the basis of its scientific merit.

☐ Review conducted by external agency.

☐ Governmental Agency: Please specify the reviewing agency or board Federal agency (e.g., NIH, NSF, USDA, etc.) and evidence of approval

☐ Nongovernmental agency (e.g., University review, specify if other):

☐ Departmental Review: I assure that this project has been reviewed and approved for scientific or instructional merit by:

☐ Expert reviewer (Name)

☐ Departmental Committee Review (Committee Name and Chairperson):

☐ Other (Describe)

Signature: _____ 1/25/2019

Department Head/Other Authorized Departmental Designee Date

XII. REVIEWED AND APPROVED BY THE IACUC

Signature: _____ 01/25/2019

Institutional Animal Care and Use Committee Chair Date

Signature: Lisa Eckert 01/25/2019

Institutional Animal Care and Use Officer Date

Following action on this application, copies of approval or denial letters will be sent to the applicant, Department Head, and appropriate College Dean.